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Immobilized membrane vesicle or proteoliposome affinity chromatography. Frontal analysis of interactions of cytochalasin B and D-glucose with the human red cell glucose transporter.

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Human red cell membrane vesicles stripped of peripheral proteins and proteoliposomes with reconstituted red cell glucose transporter (Glut1) were sterically immobilized in gel beads by freezethawing. The specific interactions between the transport inhibitor cytochalasin B (CB), D-glucose, and Glut1 were analyzed by quantitative frontal affinity chromatography. The dissociation constants, Kd(CB), for the interaction between CB and Glut1 in vesicles and proteoliposomes were similar, the average value being 92 +/- 5 nM at an ionic strength I of 0.05. Kd(CB) for Glut1 in vesicles decreased with increasing ionic strength to become 46 nM at I = 0.5. The affinity of glucose was significantly higher for Glut1 in vesicles (Kd = 24 +/- 2 mM) than for reconstituted Glut1 (Kd = 37 +/- 2 mM). The frontal analysis allowed determination of the amount of CB binding sites, which was found to be 0.33 +/- 0.06 mol per mole of Glut1 monomer (Mr = 54 000). The CB binding capacity of Glut1 in the vesicles and the proteoliposomes was stable in the presence of dithioerythritol over periods of several weeks at room temperature.

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1: Biochim Biophys Acta 1989 Jun 26;982(1):47-52

Entrapment of lipid vesicles and membrane protein-lipid vesicles in gel bead pores.

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Phospholipid vesicles were entrapped in gel beads of Sepharose 6B and Sephacryl S-1000 during vesicle preparation by dialysis. Egg-yolk phospholipids solubilized with cholate or octyl glucoside were dialysed together with gel beads for 2.5 days in a flat dialysis bag. Some vesicles were formed in gel bead pores and vesicles of sufficient size became trapped. Red cell membrane protein-phospholipid vesicles could be immobilized in the same way. Non-trapped vesicles were carefully removed by chromatographic procedures and by centrifugation. The amount of entrapped vesicles increased with the initial lipid concentration and was dependent on the relative sizes of vesicles and gel pores. The largest amount of trapped vesicles, corresponding to 9.5 mumol of phospholipids per ml gel, was achieved when Sepharose 6B gel beads were dialysed with cholate-solubilized lipids at a concentration of 50 mM. In this case the vesicles had an average diameter of 60 nm and an internal volume of 15 microliters/ml gel. The amount of vesicles trapped in Sephacryl S-1000 gel beads upon dialysis under the same conditions was smaller: 2.2 mumol of phospholipids per ml gel. Probably most of the gel pores were too large to trap such vesicles. Larger vesicles, with an average diameter of 230 nm, were entrapped in the Sephacryl S-1000 matrix in an amount corresponding to 3.0 mumol phospholipids per ml gel upon dialysis of the gel beads and octyl glucoside-solubilized lipids at a concentration of 20 mM. The internal volume of these vesicles was 22 microliters/ml gel. The yield of immobilized phospholipids was up to 19%. The entrapped vesicles were somewhat unstable: 9% of the phospholipids were released during 9 days of storage at 4 degrees C. By the dialysis entrapment method vesicles can be immobilized in the gel beads without using hydrophobic ligands or covalent coupling.

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